Interference Between Bacterial Viruses

I. Interference between Two Bacterial Viruses Acting upon the Same Host, and the Mechanism of Virus Growth*†

M. Delbrück and S. E. Luria‡

From the Departments of Physics and Biology, Vanderbilt University, Nashville, and from the Bacteriological Laboratories of the Department of Surgery, and the Department of Cancer Research, College of Physicians and Surgeons, Columbia University, New York.

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Introduction

The growth of a bacterial virus (Bacteriophage), occurring only in the bacterial cell, may be said to proceed behind a closed door. The experimenter can follow the virus up to the moment it enters the cell, and again after liberation from the cell. There is, as yet, no way of telling what goes on within the cell, except by circumstantial evidence which covers the entry of the virus into the host, its time of stay, its exit, and, perhaps, the metabolism of the host cell.

By the desire to gain more direct insight into the intracellular processes of virus growth, the present authors were led to try the simultaneous action of two different viruses upon the same host cell. There was a possibility that one virus might lyse the cell, while the other was still growing. Thus, an intermediate state of virus growth would be revealed. This expectation did not materialize. Instead, a striking case of interference was discovered, which could be analyzed in some detail. This will be reported in the present paper.

The growth of each virus alone in the host cell was also studied. Taken together, these results permit some conclusions concerning the mechanism of virus growth.

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Our attention was drawn to previously described cases of interference in animal and plant viruses (1, 2, 3, 4). We believe that the case of interference between bacterial viruses, which we describe in this paper, may help to clarify the general problem of virus interference.

Experimental Technique, Material and Procedure

For the interpretation of the experimental results a detailed acquaintance with the material used, with the principles underlying the experimental procedures, and with the quantitative analysis of the results, is indispensable. Therefore, after a brief description of the routine technique, we will discuss these items in detail.

Technique

Medium for liquid cultures: Difco nutrient broth (8 g./liter) + 0.5 per cent NaCl.

Solid medium: 1 per cent powdered agar in the above broth.

Bacterial assay by colony count. 0.1 cc. of a suitable dilution is spread, or "plated," on agar in 10 cm. Petri dishes. The colonies are counted after 24 hours incubation at 36.5°C.

Virus assay by plaque count. Suitable dilutions of the unknown are mixed with a heavy suspension of bacteria from a 24-hour slant. 0.1 cc. of the mixture is spread on agar. The virus produces holes or "plaques" in the bacterial film. Each plaque is a colony of virus particles, which has grown from a single infective center. The method is the precise analogue of the bacterial colony count method. An infective center may be either a particle of virus or a bacterium infected with virus (5).

All platings for bacterial and virus assay are made in duplicate.

Adsorption measurements are made by centrifugation of 1 cc. of a diluted sample of the mixture of virus and bacteria (four minutes in a universal centrifuge, at 3600 r.p.m.) and comparison of the amount of virus originally present with that remaining in the supernatant.

All broth cultures are kept in water bath at 36.5°C. and are continuously aerated by bubbling sterile filtered air through the cultures. This also ensures uniform mixing of the culture.

Material

Two different viruses, α and γ, were used, both of which are active upon a common bacterial host, B (*E. coli*).[1]

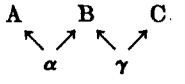
[1] These strains were kindly sent to the junior author by Dr. J. Bronfenbrenner. Originally virus α was called P28, and virus γ was called PC. Our choice of names, an adaptation to our experiments, will presently be justified. Virus γ (PC) has been purified and described by Kalmanson and Bronfenbrenner (6).

On agar, α produces large plaques (0.5–2 mm. diameter), visible after 6 hrs. incubation. After 24 hrs. incubation they are surrounded by a large halo. γ produces small plaques (0.2–0.5 mm.) visible after 24 hrs. incubation. The differences between the two viruses will be discussed in a later section. They differ markedly both in size and structure (12).

Bacterial "indicator strains" are needed in order to follow the growth of each virus in cultures in which both are present with the host. These indicator strains were obtained by the following method. It is a well known fact that the lysis of a bacterial culture is rarely complete. Usually a few hours or days after the first clearing, a secondary growth arises, which can be isolated in pure culture. This new strain is usually resistant to the action of the virus in the presence of which it arose. The sensitivity of such a variant to other viruses may be the same as that of the primary strain. Accordingly, two variants, A and C, of our bacterial host B, were obtained from secondary growths after the action of γ and α. Strain A was found to be unchanged in its sensitivity to α and completely resistant to γ, and conversely, strain C was found to be unchanged in its sensitivity to γ and completely resistant to α. Adsorption experiments showed that strain A does not adsorb γ and strain C does not adsorb α.

Sometimes, secondary cultures are lysogenic, i.e., they are carriers of the virus in the presence of which they have been isolated. A and C were tested for lysogenicity. Diluted bacterial cultures as well as their filtrates were plated with the sensitive strain B; no plaques were obtained. The possibility of their being lysogenic was thus excluded.

The relationship between the bacterial strains and the viruses may be summarized in the following scheme, in which the arrows indicate sensitivity. The symmetry of this scheme is the justification for our choice of names.



When a mixture of the two viruses is plated on agar with each one of the three bacterial strains, the plates seeded with A show only the large plaques of α, those seeded with C show only the small plaques of γ, and the plates seeded with B show both. The plates from such an experiment are shown in Fig. 1.

The system shown in the above diagram enabled us to study the growth of α and γ on the common host, both separately, and under conditions of mixed infection.

The Procedure for Virus Growth Experiments

The purpose of the growth experiments is to obtain information about a certain number of measurable quantities, which characterize the "life-cycle" of the virus in a sensitive host (7).

The first step of this life-cycle in a mixture of virus and bacteria is the adsorption of the virus on the sensitive bacterial cells. This will sometimes be referred to as the "infection" of the bacteria. The adsorption rate is measured by assaying at suitable intervals the virus in the supernatant after centrifugation of the mixture. Under definite experimental conditions (physiological state of the bacteria, temperature, medium) the adsorption rate is proportional both to the virus and the bacterial concentration (8, 9, 10). This relationship is of fundamental importance for the design of growth experiments, since it shows that the adsorption rate can be reduced, at any desired moment, to any desired extent simply by a dilution of the reacting mixture of virus and bacteria. For instance, suppose we have, in a certain mixture, an initial adsorption of 50 per cent of the virus in five minutes. If the mixture is diluted 1:1000, the relative adsorption rate will be reduced by the same factor and further adsorption will proceed at the negligible rate of 1 per cent in 100 minutes. By a heavy dilution at the proper moment one can, therefore, terminate the adsorption period, measure the amount of adsorption obtained, and study the destiny of the bacteria infected.

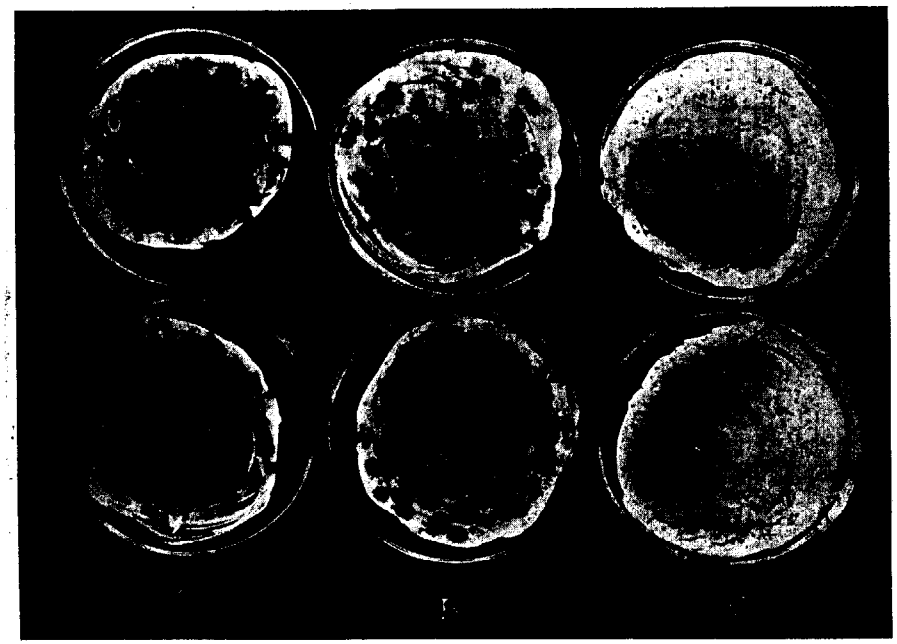


FIG. 1. Mixture of viruses α and γ plated with the three bacterial strains A, B, C. The large plaques are colonies of virus α, the small of virus γ. Strain A is the indicator strain for virus α, strain C for virus γ.

The second phase of the life-cycle is the multiplication of the virus in the cell. After the bacterial cell has adsorbed a virus particle, it retains normal appearance for a while, then, suddenly, the newly formed virus particles are liberated. In most cases the cell is lysed at the same moment. Therefore, if one follows the number of infective centers by the plaque-counting technique, one finds a period, the *constant period*, in which apparently nothing happens, and a second period, the *rise period*, in which the plaque count rises sharply. After this, the newly liberated virus particles will become adsorbed to other bacteria still present in the culture, unless this is avoided by previous high dilution of the culture, as explained above. The average number of virus particles liberated from an infected cell will be called the *burst size*.

1. The method for determining quantitatively the elements which characterize the life-cycle (adsorption, constant period, rise period, burst size) will now be described by discussing one growth experiment of the type used throughout this work.

Table I is the schedule of a growth experiment called *one-step growth* (5), because one isolates in this experiment one step in the growth of the virus, namely the step of the liberation of virus from the bacteria infected during a short initial adsorption period. The essential element of the schedule is a heavy dilution of the mixture of bacteria and virus after a few minutes of contact. By this dilution one achieves two aims, viz., one limits infection to a period which is small compared to the constant period, and one avoids the complicating effects of reinfection of the remaining bacteria by the virus liberated.

The results of the experiment given in Table I may be analyzed in the following manner.

The titers (in units per cc.) of bacteria or of virus are obtained by multiplying the colony count or the plaque count for the 0.1 cc. samples by ten times the factor of dilution.

The bacterial assay shows that the experimental bacterial culture B_{exp} contained 5×10^7 B/cc. two minutes before the virus was added. The stock virus α had a titer of 3.25×10^9 particles/cc. At the time zero, 0.2 cc. of the stock was added to 20 cc. of B_{exp} to form the adsorption mixture B_α. Therefore this mixture contained

5×10^7 bacteria/cc.
3.25×10^7 virus particles/cc.

At the time five minutes, B_α was diluted 1:2000 to form the growth-tube I, and 1:200,000 to form the growth-tube II. Further adsorption in these growth-tubes is negligible. The assay of the supernatant from the centrifuge tube shows that at this time the tube B_α contained

1.70×10^7 unadsorbed virus particles/cc.

Therefore, 1.55×10^7 virus particles/cc. had been adsorbed. This is 48 per cent of the input, and gives an average of

0.3 virus particles adsorbed per bacterium.

TABLE I

Schedule of Experiment No. 6. One-step Growth Experiment of the Virus α

Time (minutes)	
−150	0.05 cc. of a 24 hrs. broth culture of B is inoculated into 20 cc. broth, and incubated at 37°C. with aeration. This is the experimental culture, tube B_{exp}.
−10	Assay of the stock virus α: 0.1 cc. of dilution $1/10^6$ plated with bacteria washed from 24 hrs. slant. *1—337 plaques 2—316 "
−2	Assay of B_{exp}: 0.1 cc. of dilution $1/(5 \times 10^4)$ plated. *3— 94 colonies 4—104 "
0	0.2 cc. of stock virus α added to B_{exp}. This is the adsorption mixture, tube B_α. The time 0, at which virus and bacteria are mixed, marks the beginning of the timing from the virus growth.
5	0.02 cc. of B_α added to 2 cc. of broth at 37°C. in a centrifuge tube.
	From this tube
	1 cc. is added to 19 cc. of broth, and aerated at 37°C. This is the first growth-tube, I.
	0.1 cc. from tube I added to 9.9 cc. of broth at 37°C. This is the second growth-tube, II.
	1 cc. is centrifuged. Supernatant diluted 1/100, and 0.1 cc. plated with bacteria. *5—166 plaques 6—174 "
	Samples from tubes I and II taken at intervals, diluted 1/10 and 0.1 cc. plated with bacteria for virus count.
8	*7— 143 plaques 8— 151 "
11	9— 129 " 10— 136 "

* Serial numbers of the Petri plates.

The operation of sampling, mixing with bacteria, and plating two plates takes about 50 seconds. The timing is so arranged that the plating of the first plate coincides with the schedule time.

TABLE I—*Continued*

Time (minutes)		
15	11—1500 plaques 12—1400 "	
17		*13— 47 plaques 14— 44 "
19		15— 66 " 16— 62 "
22		17— 89 " 18— 83 "
25		19—106 " 20— 91 "
27		21— 89 " 22— 94 "
30		23—109 " 24— 85 "
41		25— 95 " 26— 86 "
50		27— 90 " 28—114 "

Since this is small compared to unity, only a negligible fraction of the bacteria had adsorbed more than one virus particle, and only about one-third of the bacteria were infected.

Owing to the dilution, the initial titers of bacteria and of virus in the two growth-tubes were:

	Tube I	Tube II
Bacteria/cc	2.5×10^4	2.5×10^2
Virus particles/cc	1.62×10^4	1.62×10^2

The later assays from these tubes, that is, the titers of infective centers (free virus particles + infected bacteria) after different times, are plotted in Fig. 2, in which this experiment is represented by the open circles. The plaque titer is plotted against time, relative to the initial titer. Such a plot we shall call a *One-step growth curve*. It is seen that the titer stays constant for 13 minutes. During this time the virus grows in the cell, but is not released from it. At 13 minutes the titer begins to rise and increases in ten minutes by a factor of 62.

This part of the growth curve represents the liberation of virus from the cells, which in this case are also lysed during this interval. All the cells that liberate virus in this interval were infected during the initial adsorption period. After the ten-minute rise period the titer again remains constant. This is, as ex-

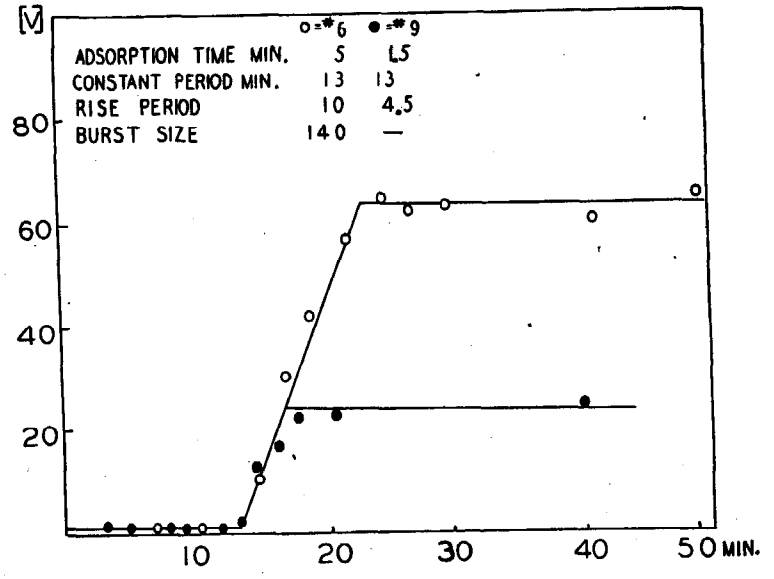


FIG. 2. One-step growth curves of virus α, single infection. ○ = experiment no. 6. ● = experiment no. 9. [V] = relative titer of virus.

plained above, because of the high dilution in the growth-tubes, which prevents readsorption.

We can now list the quantitative results:

Constant period..... 13 minutes
Rise period..... 10 minutes
Step size (final titer:initial titer)..... 62

The step size does not represent the yield of virus per infected bacterium. It has to be corrected for the fraction of virus which was not adsorbed during the initial adsorption period and which, therefore, had no chance to infect a bacterium and to grow. This fraction has to be subtracted, both from the initial and from the final titer.[2] Thus the average yield of virus per bacterium or the

$$(1)\qquad \text{average burst size} = \frac{\text{final virus} - \text{initially unadsorbed virus}}{\text{initial virus} - \text{initially unadsorbed virus}} = \frac{(184 - 1.70) \times 10^7}{(3.00 - 1.70) \times 10^7} = 140.$$

[2] For the initial titer the values obtained from the stock assay and from the assays during the constant period are averaged.

The above is an example of the typical experiment of virus growth. Several modifications of it have been used, which must now be described.

2. The most important modification is that of "multiple infection" in contradistinction to "single infection."

We have seen that the ratio of the number of adsorbed virus particles to the number of bacteria gives us the average number of virus particles adsorbed per bacterium (0.3 in the above example). If the bacteria are greatly in excess, practically no bacteria will be infected by more than one virus particle, and we speak of "single infection." On the other hand, we have seen that the rate of adsorption is proportional to both the concentrations of virus and of bacteria. Therefore, if we keep the concentration of bacteria constant, and increase the concentration of virus, adsorption will increase in direct proportion. A limiting factor might be the saturation of the bacterial cells with virus. Such a saturation phenomenon has been observed, but only at much greater concentrations than any used in our present experiments (11).

Under conditions of multiple infection several new quantitative elements become important. The first of these is the multiplicity of infection:

multiplicity = virus particles adsorbed/bacteria.

The second is the number of uninfected bacteria. If, on the average, n virus particles are adsorbed per bacterium, the fraction of uninfected bacteria will be, according to Poisson's law, equal to e^{-n}. For instance, if n equals four, there will be $e^{-4} = 1.8$ per cent uninfected bacteria:

uninfected bacteria per cent = $100e^{-n}$ (n = multiplicity).

A possible limitation of this calculation will be discussed later with experimental data.

In multiple infection experiments, the plaque count titer drops during the initial adsorption period, because nearly every bacterium collects several virus particles, but produces only one plaque. For this reason the burst size, i.e. the yield of virus per infected bacterium, cannot be evaluated by the method described in the case of single infection. Let us call

I = Input of virus
U = Unadsorbed virus
F = Final titer of virus
B = Bacteria initially present
B_i = Initially infected bacteria
P = Plaque titer during the constant period.

The burst size is then by definition

$$(F - U)/B_i.$$

The first four and the sixth of the above quantities are directly determined during the experiment. The fifth one has to be obtained indirectly. In the case of single infection, it is determined as

$$B_i = I - U,$$

since every adsorbed virus particle will infect a different bacterium; the burst size is then

$$(F - U)/(I - U).$$

The formula was used in the example on page 118.

In the case of multiple infection, B_i will be smaller than $I - U$, since the bacteria have adsorbed more than one virus particle each. On the other hand, if the multiplicity of infection is rather high, practically all the bacteria will be infected, and B_i can be replaced by B. Therefore the burst size becomes

$$(F - U)/B.$$

The value of B is usually measured a few minutes before the beginning of the experiment, and has to be corrected for the small increase between this time and the average time of adsorption. In most cases this correction, not exceeding 10–20 per cent, could be neglected, since it is of the same order as the sampling errors.

P, the plaque titer during the constant period, must be equal to the sum of the titers of infected bacteria and of unadsorbed virus:

$$P = U + B_i, \text{ or}$$
$$P = U + B \text{ if all bacteria are infected.}$$

Let us illustrate these considerations by an example.

Experiment 28. Virus γ on B, multiple infection.

$I = 17.0 \times 10^8$/cc.
$B = 0.67 \times 10^8$/cc.
$U = 6.8 \times 10^8$/cc.; $I - U = 10.2 \times 10^8$/cc.
multiplicity = 15.

$B + U = 7.47 \times 10^8$/cc.
$P = 7.5 \times 10^8$/cc.
$F = 175.0 \times 10^8$/cc.
Burst size = $(F - U)/B = 250$.

3. Experiments on mixed infection of bacteria with both viruses are carried out as those described above, except that samples are alternately plated with the indicator strains A and C, in order to obtain separate growth curves for α and γ. The analysis of these experiments will be considered in connection with experimental results.

4. In some experiments it was desired to follow the growth of the uninfected bacteria parallel to the growth of the virus. Samplings were made for colony count assays and plated after suitable dilution. Small amounts of virus in these samples do not usually interfere with the bacterial count. Bacteria, which at the moment of sampling are infected but not yet lysed, will not form colonies.

Miscellaneous Experiments

1. The growth of the bacterial strains A, B, and C was studied by following complete growth curves, starting with very dilute suspensions in broth of bacteria from 24 hour aerated cultures. The lag phase, the time of division during the logarithmic phase, and the saturation titer were calculated in the usual way from such growth curves.

2. Microscopic observations of the living bacteria, both infected and uninfected, were made by spreading a suitable dilution of the culture on the surface of nutrient agar Petri dishes, covering with a coverslide, and observing with oil immersion objective. Periodical observations of the microscopic field (generally at room temperature) were recorded on hand drawn maps.

Experimental Results

1. Growth of the Bacterial Strains A, B, and C

It was necessary, for the virus growth experiments, to work with a reproducible standard phase of the growth cycle of the bacterial strains. The bacterial growth was studied in cultures incubated with continuous aeration, and was found to be quantitatively reproducible. The results are summarized in Table II. The division time is obtained from the slope of the growth curve in the log phase, and corresponds to the time required for a twofold increase in titer. For experiments on virus growth, bacterial suspensions should be used with a titer well below the saturation value, but at the same time, as high as possible, in order to give good adsorption of virus in a few minutes. Bacterial concentrations between 3 and 10×10^7/cc. satisfied these conditions.

TABLE II

Growth of the Bacterial Strains

Strain	Lag period	Division time in the logarithmic growth phase	Maximum titer in the stationary phase
	hours	*minutes*	*B/cc.*
A	1.3	26	3.8×10^9
B	1.5	19	4.3×10^9
C	1.3	19	2.1×10^9

It is worth noting that the division time of the indicator strain C is the same as that of the primary strain B, whereas that of the indicator strain A is considerable longer (26 minutes as compared with 19 minutes). The strain A is the one which was isolated from the secondary growth after lysis of B with virus γ.

2. Growth of the Virus α

The experiments were performed as explained in the section on procedure. Experiments with bacteria in excess (single infection), and with virus in excess (multiple infection) will be reported separately.

(a) *Single Infection.* Results are summarized in Table III. Experi-

ment 6 is the one described in detail in Table I, and in Fig. 2, which also shows Experiment 9. The adsorption in these experiments was always about 50 per cent in five minutes. In Experiment 9 the adsorption time was only 1.5 minutes. The adsorption was, therefore, small and could not be accurately determined. For the same reason, the burst size was not estimated.

It will be seen that, for growth on strain B, the constant period is accurately reproducible, with an average of 13 minutes. This value is a characteristic of the system B + α under our standard conditions. We will see later that it is not changed by multiple infection.

The rise period is fairly reproducible (7.5 to 10 minutes) in different experiments with the same adsorption time of five minutes. When only 1.5 minutes are allowed for adsorption, the rise period is reduced to four minutes. This is proof that the length of the rise period is, in part, determined by the length of the adsorption period. Earlier infected bacteria liberate the virus earlier. The true variability of the period between adsorption and liberation of virus is given by the difference between the rise period and the adsorption period. It is, therefore, only about three minutes and a half. The value thus obtained will be called the "true rise period." The burst size, *i.e.*, the average yield of virus particles per infected bacterium, is also well reproducible, with an average of 142.

TABLE III

Growth of Virus α. Single Infection Experiments

Experiment	Adsorption time	Adsorbed virus	Constant period	Rise period	True rise period	Burst size
No.	*minutes*	*per cent*	*minutes*	*minutes*	*minutes*	
6	5	45	13	10	5	140
7	5	58	13	9	4	144
8	5	*	12.6	7.5	2.5	*
9	1.5	*	13	4.5	3	*
Average			13		3.6	142

* Not measured.

(b) *Multiple Infection.* The results are given in Table IV.

It will be seen that the adsorption rate is the same as in the experiments with single infection. This means that, even for the highest multiplicities attained in these experiments, the bacterial surface is still well below the point of virus saturation.

The constant period is precisely the same as that for single infection, 13 minutes. The rise period is shorter, if one compares experiments with equal adsorption periods. This is to be expected because, with virus in excess, practically all the bacteria will be infected at least once within a very short time, probably within the first minute.

The burst size is larger than for single infection (203 as compared with 142), a difference well outside the limit of experimental errors. The burst size does not show any correlation with the multiplicity of infection.

The increase in burst size may have the following simple explanation. Let us suppose that the facteria can still divide after infection almost until lysed. In multiple infection, both daughter cells of such a division will, in general, be infected, whereas in single infection only one of them may be infected. In multiple infection the actual number of infected bacteria will, therefore, be larger than the number given by the bacterial assay, and therefore the total yield of virus will be increased proportionally. Quantitatively, this explanation runs as follows: the burst size is increased by 45 per cent; this would call for an increase in the number of bacteria of 45 per cent. Such an increase would require 10 minutes, just three minutes less than the constant period of virus growth. We would have to assume, then, that bacteria can divide until three minutes before lysis.

TABLE IV

Growth of Virus α. Multiple Infection Experiments

Experiment	Adsorption time	Adsorbed virus	Multiplicity of infection	Constant period	Rise period	Burst size
No.	*minutes*	*per cent*		*minutes*	*minutes*	
12	5	49	6	13	7	220
13	5	45	9	13	4.5	175
14	3	50	11.5	12.5	4	215
Average				13	5	203

(c) *Microscopic Observations.* Bacteria, multiple infected with virus α, were transferred, shortly before the end of the constant period, to agar plates and were observed under the microscope. The first cells were lysed about fifteen minutes after infection and all were lysed 28 minutes after infection. The slight delay of the onset of lysis, as compared to the onset of virus liberation (a delay of about two minutes), can probably be ascribed to the lower temperature at which the observation takes place.

3. Growth of the Virus γ

(a) *Single Infection.* Results are summarized in Table V. One experiment is shown in Fig. 3 (solid circles).

The rate of adsorption for virus γ is a little higher than for virus α. 70–80 per cent is adsorbed in five minutes.

TABLE V

Growth of Virus γ. Single Infection Experiments

Experiment	Adsorption time	Adsorbed virus	Constant period	Rise period	True rise period	Burst size
No.	*minutes*	*per cent*	*minutes*	*minutes*	*minutes*	
19	5	80	21	8	3	140
21	5	75	21	9	4	130
18	3	45	21	9	6	135
22	1.25	18	21.5	5	3.75	*
Average			21		4.2	135

* Not measured.

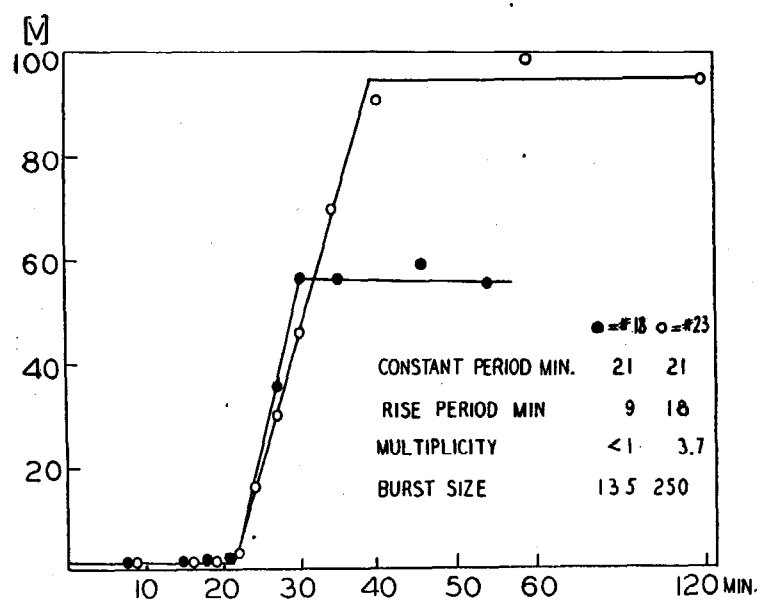


FIG. 3. One-step growth curves of virus γ. ● = experiment no. 18, single infection. ○ = experiment no. 23, multiple infection. [V] = relative titer of virus.

The constant period is 21 minutes and is again accurately reproducible.

The rise period depends on the adsorption time, as with virus α. The true rise period (the variability of the period between infection and virus liberation) is about four minutes.

The burst size is well reproducible, with an average of 135. The difference between this value and the one found for virus α, 142, is within the limits of experimental error. In the case of virus γ, however, the calculation of the burst size is not quite unambiguous, because the plaque titer does not always stay accurately constant after the main increase has occurred. A slow continuous rise, amounting to about 20 per cent, may follow the first steep rise. This slow rise of the titer may be due to a small amount of readsorption on previously uninfected bacteria in the growth-tubes. The burst sizes given above are calculated from the titers at the end of the steep rise, disregarding the further slow increase.

(b) *Multiple Infection.* Results are given in Table VI. Experiment 23 is shown in Fig. 3 (open circles).

TABLE VI

Growth of Virus γ. Multiple Infection Experiments

Experiment	Adsorption time	Adsorbed virus	Multiplicity of infection	Constant period	Rise period	Burst size
No.	*minutes*	*per cent*		*minutes*	*minutes*	
23	5	65	3.7	20.5	18	250
24	5	61	6.3	21	13	250
28	5	60	15	23	16	235
30	5	65	15	22	20	315
Average				21.5	16.5	262

The adsorption rates and the constant period are the same as for single infection, as in the case of virus α.

The rise period, which in the case of virus α, was, as expected, shortened by multiple infection, is in this case considerably lengthened. We are unable to give a plausible explanation of this result. We suspect that it is in some way connected with the fact that the constant period for this virus is longer than the division time of the bacteria.

The average burst size is 262, an increase of about 100 per cent over the burst size for single infection. As in the case of the other virus, the burst size shows no correlation with the multiplicity of infection. Here, too, the increase may be due to the division of multiple infected bacteria. The division of the bacteria would have to continue until a few minutes before virus liberation, in order to account for the large increase.

(c) *Microscopic observations* show a strict correlation between virus liberation and lysis, both with regard to the onset and completion of these two processes. All bacteria are lysed within the expected time limits.

4. The Survival and Growth of the Uninfected Bacteria in the Growth Tubes

The purpose of these experiments was twofold. First, to see whether the titer of viable bacteria, in a mixture of bacteria and virus, drops in proportion to the infection of the bacteria. Second, to see whether the uninfected bacteria continue to grow while the virus titer remains constant after virus liberation. Most of these experiments were done with virus γ, and the results of these will be given first.

(a) *Virus* γ. In two experiments of the one-step growth type with virus in excess, the bacterial titer was followed by platings parallel to the virus titer platings. The following facts were observed:

1. An initial decrease of the bacterial titer, as expected on the hypothesis that infected bacteria are eliminated by lysis.

2. A slight retardation in the division rate of the uninfected bacteria, lasting between 20 and 40 minutes. Control experiments with bacteria in the absence of virus, showed the same retardation. It is, therefore, to be ascribed to manipulation of the culture, when it is transferred, diluted, etc.

3. Normal growth of the uninfected bacteria after this period, throughout the remainder of the experiment.

It was desired to check whether the initial diminution of the bacterial titer is in quantitative agreement with the theoretical expectation. The fraction of uninfected bacteria according to Poisson's formula, should be e^{-n}, where n is the multiplicity of infection, *i.e.*, the average number of virus particles adsorbed per bacterium. The applicability of this formula depends upon two conditions. First, it must be assumed that the bacteria are all equal in their affinity toward the virus. Second, the plaque count titer must not only be proportional to the number of virus particles in the suspension, but must be actually equal to it, *i.e.*, the efficiency of plating (5) must be unity.

An experiment was performed, in which different amounts of virus were added to the same number of bacteria, the adsorption was measured, and the surviving fraction of bacteria determined by colony count.

Results of the whole group of experiments are summarized in Table VII.

It will be seen that the calculated and the experimental titers of the surviving bacteria are similar in all cases; in some cases they agree closely. The deviation between experimental and theoretical values is greatest for high multiplicity of infection, the experimental titer in these cases being smaller than the expected values. Deviations can be accounted for by assuming that the true virus titers are not more than 20 per cent higher than the values given by plaque assays.

(b) *Virus* α. Only one growth curve was followed with bacterial and virus assays in parallel. The initial decrease of the bacterial titer was of the expected magnitude. The growth of the uninfected bacteria, after a slight retardation, continued normally as in the case of virus γ.

These results confirm our picture of the infection of the bacteria by the virus particles and of the elimination of infected bacteria by lysis. Moreover, they justify the use of Poisson's formula for the calculation of the fraction of uninfected bacteria. This will be used frequently in the experiments reported in the next section.

TABLE VII

Survival of Bacteria in the Presence of Virus γ

Experiment	Initial bacterial concentration	Adsorbed virus	Multiplicity of infection	Surviving bacteria	
				Experimental	Calculated
No.	*B/cc.*	*V/cc.*		*per cent*	*per cent*
25a	8.0×10^7	1.9×10^7	0.24	75	79
25b	8.8×10^7	6.0×10^7	0.68	30	49
25c	8.3×10^7	23.5×10^7	2.9	2.7	5.5
26	8.0×10^7	15.5×10^7	1.9	12.5	15
27	7.3×10^7	29.0×10^7	4	0.7	1.8

5. Mixed Infection of Bacteria with Virus α *and Virus* γ

A variety of experiments were made with different ratios between the bacterial and virus titers. It may be well to state at the outset that there was never found any interference between the two viruses as regards *adsorption*. The adsorption rates for both viruses α and γ were found the same, or nearly the same as in the experiments with either virus alone.

(a) *Mixed Infection with Both Viruses Greatly in Excess.* An experiment of this type is shown in Fig. 4. The bacteria were mixed at time zero with both viruses in excess, such that, at the end of the adsorption period, the multiplicity of infection was $(4.2\alpha + 4.3\gamma)$/bacterium.

It will be seen that there is hardly any increase of virus α, whereas the increase of virus γ takes place as in the absence of virus α (constant period 21 minutes, rise period 15 minutes, burst size 220). The amount of virus α found in the first samples is equal to the measured amount of *unadsorbed* virus α. Later, it is increased

only by a factor 1.5. In the absence of virus γ, there would have been an increase of the titer of virus α by a factor of 40–50 in this experiment. The small increase of virus α which does take place is completed at 17 minutes, well before the beginning of the rise of the γ-titer.

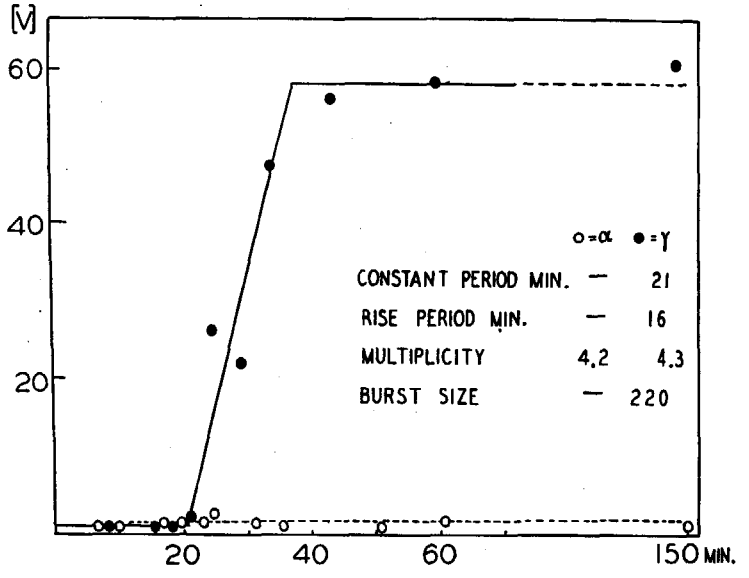


FIG. 4. Experiment no. 34. Mixed infection. Bacteria plus viruses α and γ in large excess. O = virus α. ● = virus γ. [V] = relative titer of virus.

The experiment suggests that there is interference between γ and α, such that mixed infection of a bacterium by γ and α leads to the suppression of virus α.

(b) *Mixed Infection with Both Viruses Slightly in Excess.* In the experiment shown in Fig. 5, the mixture of bacteria and viruses was set up in such proportions, that at the end of the adsorption period the multiplicity of infection was only $(1.55\alpha + 1.16\gamma)$/bacterium. Under these conditions, a sizeable fraction of the bacteria will not be infected with virus γ, namely $e^{-1.16} = 31$ per cent, using Poisson's formula.

Fig. 5 shows that in this case there is, besides the normal increase of virus γ after 21 minutes, also an increase of virus α between 13 and 17 minutes. This increase is smaller than the increase that would have occurred in the absence of virus γ, and it is not accompanied by a simultaneous increase of the γ-titer. Calculation shows that the increase is three times smaller than that expected in the

absence of virus γ. Since just 31 per cent of the bacteria were not infected by virus γ, the increase of virus α can be ascribed to its growth in these bacteria. Evidently, only the bacteria that were infected with virus α and free of virus γ, have liberated the virus α.

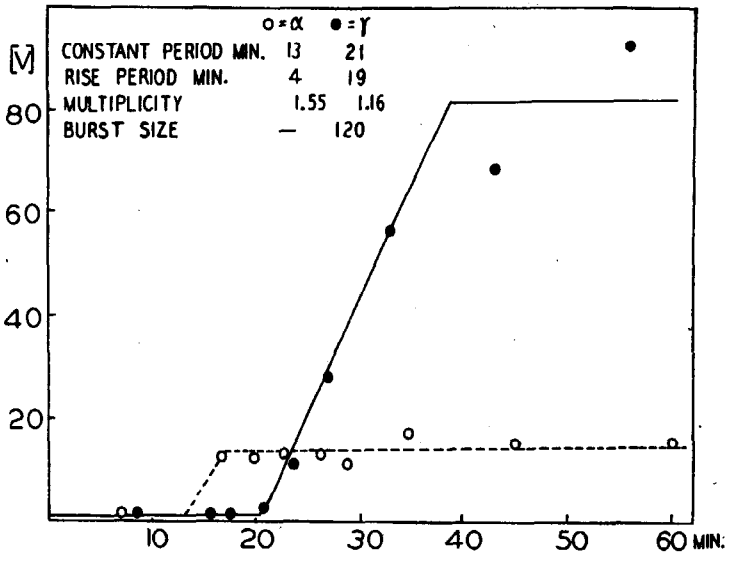


FIG. 5. Experiment no. 32. Mixed infection. Bacteria plus viruses α and γ in slight excess. O = virus α. ● = virus γ. [V] = relative titer of virus.

Regarding interference we conclude that a bacterium infected with viruses α and γ will liberate only virus γ, after a latent time equal to the constant period of virus γ. No growth of virus α takes place in these bacteria.

Going back to experiment (*a*), we can verify that the small increase of virus α found in that case corresponds almost exactly to the liberation of virus α from the few bacteria which had adsorbed virus α and not virus γ (1.5 per cent by Poisson's formula).

(c) *Inactivation of Virus α When Adsorbed on γ-Infected Bacteria.* Let us consider a bacterium which has adsorbed both viruses α and γ, and which is plated with the bacterial strain A (sensitive to virus α only) before the liberation of virus γ has taken place. The bacterium should not produce a plaque if no liberation of virus α takes place.

In the two experiments of Figs. 4 and 5, calculation showed an initial

diminution of the titer of infective centers of virus α, when compared with the titer to be expected in the absence of virus γ. This diminution proves that a virus α particle which is adsorbed on a bacterium infected with virus γ, is actually lost. If it were liberated when the liberation of virus γ takes place, it could infect a bacterium A, and produce a plaque.

The following experiment was designed to prove this point under conditions permitting a more sensitive quantitative test. Bacteria were mixed with a large excess of virus γ and a small amount of virus α. Under these conditions practically

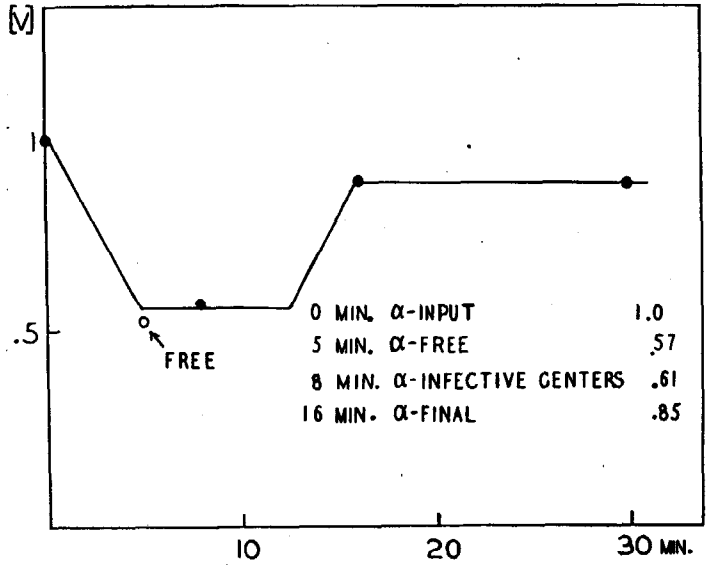


FIG. 6. Experiment no. 42. Mixed infection. Bacteria plus a large excess of virus γ and a small amount of virus α. [V] = relative titer of virus α.

all the bacteria will be infected with virus γ, and any virus α which gets adsorbed should be lost. The remaining titer of α plaques should be equal to the titer of unadsorbed virus α.

The experimental results are given in Fig. 6. It shows the decrease of the titer of virus α, substantially to the amount of unadsorbed virus. Here, too, the small increase of virus α after 13 minutes is due to growth on the few bacteria which escaped infection with virus γ.

Some experiments were designed to find the limitations of the suppression of the growth of virus α by virus γ.

(d) *Mixed Infection with Virus α in Excess of Virus γ.* Experiments were done to test whether suppression takes place when virus α is present in greater amount than virus γ. In one such experiment, the multiplicity of infection was $6.3\alpha + 2.1\gamma$; in another it was $3.5\alpha + 1.4\gamma$. In both cases the suppression of virus α was evident. A small increase of virus α was quantitatively accounted for by the growth on bacteria which had not adsorbed any particle of virus γ.

(e) *Infection with Virus α Preceding Infection with Virus γ.* It might be expected that the suppression would be less complete if virus α is given several minutes start in its attack upon the bacterium. The results of a series of such experiments are listed in Table VIII.

TABLE VIII

Mixed Infection. Virus α Preceding Virus γ

Experiment	Interval of time between the infection with the two viruses	Multiplicity of infection		Increase of virus / Infected bacteria	
		Virus γ	Virus α	Virus γ	Virus α
No.	*minutes*				
43	2	~3	<1	*	1.8
76	4	7.7	4	124	3.5
40	4	4.3	1.4	210	9
74	6.5	3	3	62	105
71	7.5	6	4	17	130

* Not measured.

It is seen that suppression is complete if virus γ is added two minutes after virus α. The small increase of virus α, is again accounted for by growth on bacteria not infected by virus γ. With an interval of four minutes, the amount of growth of virus α, although small, is still too large to be compatible with suppression of α growth in all the bacteria infected with virus γ. With larger intervals of time, 6.5 and 7.5 minutes, suppression of α growth disappears rapidly; at the same time the increase of virus γ diminishes. In no case, however, is there liberation of virus α and virus γ from the same bacterium. This is proved by the fact that the increase of virus α always occurs between 13 and 20 minutes, before the beginning of the increase of virus γ.

It appears that the probability of virus α suppression declines rapidly, if the infection with virus γ occurs in the time interval between four and six minutes after infection with virus α.

(f) *Growth of Virus γ in Bacteria Multiply Infected with Virus α.* We have seen that the growth of virus γ is normal in bacteria infected by approximately equal amounts of the two viruses. It was interesting to find whether the same

was true when the bacteria were infected with many particles of virus α and only one particle of virus γ. Experiments were designed to test this point. Two one-step growth experiments for virus γ were run in parallel; in one of them the adsorption mixture received a large excess of virus α simultaneously with the virus γ; in the other, the control, the suspension of virus α was replaced by an equal amount of broth.

It was found that an excess of virus α did not change the length of the constant period, but did reduce the total increase of virus γ to about one half the value obtained in the absence of virus α. If the reduction of the total increase is due to a reduction of the number of bacteria which liberate virus γ, the plaque counts during the constant period should also be reduced by the addition of virus α. The experiments actually show such a reduction of about 50 per cent.

Discussion

The experiments described in the preceding sections establish the following facts.

The growth of each of the viruses follows the pattern of other well studied cases (7). The virus is first adsorbed by the bacterial cell. After elapse of a certain accurately reproducible time, a large amount of virus is released in a sudden burst, while the cell undergoes lysis. The time interval between adsorption and lysis varies little among the individual bacteria of a growing culture.

If the bacteria are simultaneously infected with several virus particles of the same kind (multiple infection), the results are the same, except for a somewhat increased yield of virus. However, this increased yield may be only apparent, and may be due to an underestimation of the number of infected bacteria which continue to divide for a few minutes after infection. We accept this explanation tentatively and conclude that the true yield of virus from an infected bacterium is the same in multiple infection as in single infection.

The quantitative results are summarized in Table IX.

A bacterium infected simultaneously by virus particles of both types (mixed infection), will liberate only one type, virus γ, 21 minutes after infection, as in the case of infection with virus γ alone. Virus α not only fails to grow, but the infecting virus too is inactivated. The suppression of the growth of virus α occurs even when this virus reaches the bacterial cell several minutes in advance of the suppressor, but it is then not quite complete. Some bacteria will then liberate virus α, but no virus γ will be released from these bacteria. They are lysed under the influence of virus α at the time which is characteristic for this virus

(13–17 minutes). *In no case will one bacterial cell liberate virus particles of both types.*

It should be noted that a *single* particle of virus γ is able to suppress completely the growth of virus α in any given bacterium. This is proved by the experiments on survival of uninfected bacteria, which showed that the plaque titer and the absolute number of particles of virus γ are in close absolute agreement.

The amount of virus γ liberated after mixed infection is normal, except when the cell has been infected with a great excess of virus α. In that case the yield of virus γ is somewhat reduced, because some bacteria fail to liberate either virus.

TABLE IX

Comparison of the Results for Virus α and Virus γ

	Constant period = minimum latent period	True rise period = variability of the latent period	Burst size = average yield of virus per bacterium
	minutes	*minutes*	
Virus α	13	3.6	142
Virus γ	21	4.2	135

We may add at this point a summary statement regarding the differences between the two viruses.

Virus α and virus γ, isolated at different times and localities, have at least one common host. When interacting separately with this host, they give plaques of different sizes. They do not induce cross immunity, i.e., the resistant secondary growth induced by either one of them is fully sensitive to the other virus. If one wishes to consider these secondary growths as strains which differ from the original one, one would say that the host range of the two viruses differs. Such close relatives as strains A, B, C may serve to differentiate between the two viruses. The physical characteristics of the two viruses α and γ are conspicuously different. This may be seen from published electron-micrographs of the two viruses (12). The pictures show striking differences both in size and structure. The difference in size is also reflected in the difference of sensitivity of the two viruses to x-rays (12, 13). Finally, differences in behavior after treatment with ultraviolet light, discussed in the next paper (20), may be mentioned. While inactivated virus γ can still suppress the growth of virus α in the bacterium and the growth of the bacterium itself, inactivated virus α shows no effects at all.

To sum up, the two viruses differ as much as any two viruses with a common host could possibly differ.

On the basis of these experimentally established facts, and of others previously found, we will now discuss the intracellular virus growth.

The following questions may be asked in this connection, some as yet inaccessible to direct experimental test, but all, probably, essential for the formulation of a comprehensive theory of virus growth:

(1) Is lysis the immediate cause of virus liberation, or is it a secondary by-effect of the infection?

(2) What determines the yield of new virus from any given cell? Is it the amount of material which is available for synthesis, or is the synthesis terminated by some other series of reactions which causes liberation and lysis after a certain time?

(3) Does multiplication proceed like that of a bacterium in a suitable growth medium, increasing from one to two to four to eight, etc., or does it proceed linearly through the intervention of some heterocatalyst from one to two to three to four, etc., or does the synthesis of all new virus occur simultaneously?

We will deal with these questions in order, proceeding from the problems with fairly direct evidence to those requiring more abstract reasoning. Although we are far from being able to construct a complete theory, the facts which have been secured help to narrow the field of speculation and suggest a scheme of interpretation of at least heuristic value.

1. Relation between Lysis and Liberation

Virus liberation and lysis occur simultaneously. Most observers have consequently pictured lysis as the immediate cause of virus liberation. The cause of lysis would then have to be sought in some by-effect of virus growth. This notion cannot explain the mechanism of virus liberation in lysogenic strains, in which lysis does not take place.

Recently, E. Cordts (14) has found evidence that lysis very probably is not the immediate cause of virus liberation. She studied a case in which virus liberation occurs in the form of a sudden burst, as in sensitive strains, but is not accompanied by lysis of the cell. In the case of this strain the cells survive infection and proceed to divide under certain conditions, namely if the medium contains more than 0.5 per cent NaCl.

This shows that virus liberation of the burst type is not the result of the lysis of the cell; the lysis appears to be rather an accessory phenomenon which may or may not accompany virus liberation. We may therefore restrict our discussion to the growth and liberation of virus, and regard lysis as unessential.

2. Burst Size, Latent Period

It is rather obvious that the length of the constant period is the time required by the cell for the synthesis of a standard number of virus particles, because:

(a) When the temperature of the growth experiment is changed, the constant period is changed in proportion to the growth rate of the bacteria, but the number of particles liberated per bacterium is unchanged (5);

(b) In our experiments on mixed infection, the liberation of virus γ occurred at the standard time reckoned from the infection of the cell with *this* virus, even when this infection had been preceded by infection with virus α.

The fact that the yield of virus per bacterium is nearly the same for both viruses, although the two viruses differ greatly in size, suggests that the number of particles synthesized is limited by the availability of some substrate, a definite amount of which enters into each virus particle, either of type α or of type γ.

The cycle of events which begins with infection and ends with virus liberation must be fairly independent of the bacterial division cycle, for these reasons:

(a) The length of the constant period of virus growth may be either shorter or longer than the bacterial division cycle, depending on the virus;

(b) Bacteria infected in different phases of the division cycle cannot differ much in the length of their respective latent periods, since the latent periods of the individual bacteria in the one-step growth curves vary but little. In these experiments the population of bacteria is a mixture of individuals in all phases of their division cycle.

We conclude, therefore, that the intracellular virus growth is limited by the availability of some substrate and that liberation takes place when the growth has run to completion.

3. Mechanism of Virus Growth

In order to draw conclusions about the growth mechanism itself, we must take into consideration the experiments on multiple and on mixed infection.

In multiple infection, the infection with the additional virus particles does not change, qualitatively or quantitatively, the course of events determined by one of them. This result is in conflict with the idea of a simple growth mechanism, like that of a bacterium in a nutrient medium, on the basis of which one would expect a shortening of the latent period in multiple infection. In mixed infection, the infection with virus α does not change the course of events determined by infection with virus γ.

In one respect, these two groups of experiments reveal a strikingly similar result. It would seem simplest to consider multiple infection as a special case of mixed infection, namely as the case in which the infecting strains are identical. Mixed infection then is the more general case, and as such reveals a new feature, namely the asymmetry in the relation of the two viruses to the host, virus γ being able to suppress virus α, and not vice versa.

The situation may be expressed in this way: the cellular function of growing virus is put into maximum operation by *one* virus particle; one virus particle saturates this cellular function. A simple hypothesis may be proposed to explain this behavior of the cell.

Hypothesis of the Key-Enzyme. The saturation may be due to the fact that among the bacterial enzymes which are necessary for virus synthesis, there is one "key-enzyme" which is completely engaged by one virus particle. Other virus particles coming later, either remain idle, or displace the first one from the key-enzyme. Thus, in multiple infection, only one particle grows; in mixed infection, virus γ displaces virus α. The key-enzyme may be just one molecule, or several, but, if several molecules, all of them must be engaged by one virus particle. When the cell divides, or rather before the cell divides, the key-enzyme must be doubled; a cell, multiple infected in this stage, would then be able to give two infected daughter cells. The incoming virus may be broken up before it engages the key-enzyme. This would explain the loss of the infecting virus α when its growth is suppressed by virus γ.

The diminution of the suppression of the growth of virus α, if this virus is given a start, is only apparently a gradual one. If the individual cell is considered, the suppression follows an "all-or-none" law: the cell either proceeds to make only virus α, or it makes only virus γ. In terms of the hypothesis of the key-enzyme this means that the virus γ particle either succeeds in displacing virus α or does not succeed. If it succeeds, then all previous reactions tending toward synthesis of virus α are frustrated, and are replaced by the reactions leading to the synthesis of virus γ.

The key-enzyme must be a common factor in the growth of the dissimilar viruses α and γ. Obviously there must be other, specific, enzymes involved in the synthesis of each type of virus. These specific enzymes are probably those by which the indicator strains A and C differ. These indicator strains do have different enzymatic machineries, since they synthesize the "receptor-spots" for only one or the other of the viruses.

In the second paper it will be shown that experiments with virus treated with ultraviolet light are easily interpreted on the basis of the hypothesis of a key-enzyme.

The hypothesis of the key-enzyme explains the results of multiple infection as a special case of interference, which may be called "self-interference". Future experiments must show the usefulness of this concept.

It should be mentioned that the results of multiple infection might be explained in an altogether different manner, by assuming that the infecting virus particles only take part in a fast initial reaction, during which the framework for the synthesis of all the virus particles to be synthesized is laid down. If there are several similar infecting virus particles, the rate of this initial reaction may be accelerated; but, if the duration of the initial reaction is short compared to the total duration of the constant period, this acceleration would not be observable. It is necessary to assume that the infecting particle participates in the initial reaction only, since otherwise the speed of the later reactions would be influenced by multiple infection, and a change of the constant period should be observable.

It is hardly possible to elaborate this hypothesis, since it is difficult to conceive a chemical mechanism by which a virus particle can lay down a course of reactions in which it does not participate.

Interference between virus γ and virus α has no obvious interpretation in this picture. It can be expressed by saying that virus γ has a stronger directing tendency in the laying down of the framework, in fact so strong a directing force that it upsets the reactions initiated by virus α and forces the cell to follow its own directions, and indeed to follow them at a rate, as if no virus α had started specific reactions previously.

The discussion has yielded answers to the questions which we posed at the beginning. We arrive at the following picture of the growth of bacterial virus.

After adsorption on the sensitive host, the virus starts reproducing,

not autocatalytically like a bacterium in a suitable medium, but with the intervention, among other enzymes, of a key-enzyme, present in limited amount, perhaps in single unit in each cell.

The liberation of virus takes place after a definite time, when the available amount of some substrate has been used up. Interference exists both between particles of the same virus and between particles of different viruses, and is to be interpreted as a competition for an enzyme rather than for substrate.

4. Interference Phenomena in Other Viruses

(a) *Bacterial Viruses.* With our two viruses, secondary growth arising after the action of each is fully sensitive to the other virus. This is not the case when viruses are related, particularly when they are adsorbed by the same bacterial antigen or "receptor-spot." In cases where secondary growth is not truly resistant, but is a carrier of the virus (lysogenic), it will in general also be resistant to the action of related viruses. This kind of interference may be similar to that described in this paper.

The best documented instance is that described by Burnet and Lush (15). These authors worked with two related viruses, C and C′, both active upon a strain SF of *Staphylococcus albus*. Virus C produces a rich secondary growth, which is lysogenic. Virus C′ produces little secondary growth, which is truly resistant. The lysogenic secondary growth produced by virus C is resistant to virus C′. This resistance to virus C′ is effective a few minutes after virus C has been adsorbed. Burnet and Lush assume that, in these few minutes, a true resistance has been induced in the bacterium. Bruce White (16), discussing this case in connection with similar observations of his own, suggested that the induced resistance is due rather to a blockade, by the indigenous virus, of the bacterial receptor spots. In view of our results, it seems possible that the blockade is not a blockade of the receptor spots, but of the key-enzyme. In our case, at any rate, we have unambiguous proof that the interference is not related to the adsorption of the viruses on the receptor spots.

(b) *Animal Viruses.* Hoskins (1) discovered an interesting case of interference between two strains of yellow fever virus, and a detailed experimental investigation of this has been published by Findlay and MacCallum (2).

The strains are the normal pantropic one, and a neurotropic one obtained by passage through mouse brain. If both these strains are injected either subcutaneously, intraperitoneally, or intracerebrally into a monkey, the animal shows only the reactions characteristic of the neurotropic strain. Similar effects were observed when these strains were tested on hedgehogs and mice.

Interference between the virus of Rift Valley fever and the two strains of yellow fever, was also studied by the same investigators. The Rift Valley fever virus is serologically unrelated to that of the yellow fever and no cross immunity is induced between these two. This instance, therefore, is analogous to ours; the only similarity between the two viruses lies in the symptomatology of the induced diseases.

It was found that neurotropic yellow fever virus protects mice against the Rift Valley fever virus; the latter in turn protects monkeys against the pantropic yellow fever virus. Different hosts had to be used, because Rift Valley fever virus produces severe symptoms in mice and mild symptoms in monkeys. This introduces a limitation in the study of interference and illustrates the desirability of working with indicator hosts.

Jungeblut and Sanders (3) described a case of interference between a murine strain and a normal strain of poliomyelitis virus which are genetically related. The murine strain, of limited pathogenicity for monkeys, can protect these animals against the normal strain.

(c) *Plant Viruses.* The extensive literature concerning interference in plant viruses has been reviewed by Price (4). The present authors are not familiar with the technique of plant virus work and, therefore, are unable to fully discuss the relation of this work to their findings. However, a few remarks may be made.

In typical cases, a plant infected with one virus will, upon infection with another "related" virus, fail to develop the symptoms normally attendant upon the second infection. This failure to develop symptoms is often called "acquired immunity." In some cases (17, 18) fairly convincing evidence that the second virus actually does not grow could be adduced, and in others this is a plausible presumption. Here, then, as in our case, the growth of one virus is suppressed by the presence of another. That suppression is confined to the areas actually invaded by the first virus has also been shown in some instances (18, 19). Naturally, since the detection of this interference depends on symptom expression, it is limited to cases in which the second virus has time to develop its symptoms before the first virus destroys the tissue. Therefore, the first virus must develop symptoms more slowly than the second, or it must develop symptoms mild enough to permit expression of the second virus symptoms. For this reason, interference tests can, in most cases, only be made in one direction. This technique would not be applicable to test, for instance, whether a necrotic virus suppresses the

growth of a mottling one. It would be necessary to recourse to indicator plants sensitive only to one of the two viruses.

Acquired immunity to one virus by infection with another has sometimes been attributed to competition for common substrates and as such has been taken as a criterion of genetic relatedness. The evidence is good, that, if the viruses are known, from other experiments, to be related, they will give mutual protection. The reverse conclusion, from protection to relatedness, seems however less secure in view of our results of interference between two very dissimilar viruses. It is true that many cases of apparent lack of interference between viruses known to be dissimilar have been reported. Our case shows that interference can nevertheless be present in only one direction in the case of unrelated viruses. In those cases of plant viruses in which relatedness has been inferred on the basis of interference proved in one direction only, the correctness of the inference may be questioned.

The following scheme may be found to fit all facts:

1. Two closely related viruses will interfere in both directions.
2. Two unrelated viruses may interfere either in only one direction, or in neither direction.

It follows that:

1. Interference in both directions proves relatedness.
2. Interference in one direction is insufficient basis for assuming relatedness.

Summary

1. The growth of two bacterial viruses active upon the same host is analyzed.

2. Multiple infection of a bacterium with several particles of the same virus has qualitatively and quantitatively the same effects as infection with a single virus particle.

3. Mixed infection of a bacterium with particles of both viruses results in complete suppression of the growth of one virus, while the other grows normally (non-reciprocal interference). This interference is studied in detail under various experimental conditions.

4. On the basis of these and of other results a theory of the growth mechanism of bacterial viruses is elaborated. Virus is considered to be produced with the intervention of a "key-enzyme," present in limited amount in each bacterial cell.

The results of the experiments on multiple infection are interpreted as interference between particles of the same virus (self-interference). Self-interference and non-reciprocal interference are jointly attributed to competition for the key-enzyme.

5. The bearing of these results on other cases of interference between viruses is discussed.

Many of the experiments described in the present paper were performed in the summer, 1941, while the authors were guests of the Biological Laboratory, Cold Spring Harbor, N. Y. The authors are greatly indebted to the Long Island Biological Association and in particular to Dr. M. Demerec, Director of the Laboratory, for the hospitality extended to them. Thanks are also due Miss Edna Cordts for technical assistance.

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